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REVIEW

Animal Studies on Medicinal Herbs: Predictability, Dose Conversion and Potential Value

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Animal studies testing medicinal herbs are often misinterpreted by both translational researchers and clinicians due to a lack of information regarding their predictability, human dose equivalent and potential value. The most common mistake is to design or translate an animal study on a milligram per kilogram basis. This can lead to underestimation of the toxicity and/or overestimation of the amount needed for human therapy. Instead, allometric scaling, which involves body surface area, should be used. While the differences in the pharmacokinetic and pharmacodynamic phases between species will inevitably lead to some degree of error in extrapolation of results regardless of the conversion method used, correct design and interpretation of animal studies can provide information that is not able to be provided by *in vitro* studies, computer modeling or even traditional use. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: medicinal herbs; dose translation; allometric scaling; conversion; equivalent; correlation.

INTRODUCTION

Animal studies on medicinal herbs are easily misinterpreted due to the lack of information regarding their predictability, potential value and human dose equivalent. One of the most common misinterpretations is to assume that isometric scaling, which involves a simple conversion by body weight, is appropriate for conversion of animal dose to humans. This type of scaling rarely correlates to the correct dose, and it is not correlated to oxygen utilization, blood volume, renal function, capacity to retain heat or most biological factors (Liu and Chen, 2001). Isometric scaling of animal studies may lead practitioners or scientists to underestimate the toxicity and/or overestimate the amount needed for therapy (Greaves *et al*., 2004). Instead, allometric scaling, which involves body surface area (BSA) should be used. Allometric scaling attempts to compensate for the fact that larger animals normally have a slower metabolic rate and therefore require a smaller drug dose on a milligram per kilogram basis (Dubois and Dubois, 1915; Valentin *et al*., 2009; Sharma and Mcneill, 2009).

The goal of this article is to review the most current information regarding potential value, dose extrapolation and predictability of animal studies on medicinal herbs. In cases where data on herbal extracts were not available, we applied relevant data from pharmaceutical studies.

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POTENTIAL VALUE

Animal studies on medicinal herbs can provide valuable information, even if the herb has been traditionally used over hundreds of years. The example of analgesic nephropathy emphasizes the importance of animal studies on herbs and or drugs that have been combined. Aspirin and extracts of Salix daphnoides *et spp*. (Willow bark) containing salicin have some inherent nephrotoxicity (Schwarz, 1993; Molland, 1978). Likewise, phenacetin, which was introduced in 1887, is associated with slight amounts of nephrotoxicity (Whelton, 1999). However, when the two are combined and used over a number of years, as was common in the first half of the 20th century in Europe, Australia and the United States, they cause analgesic nephropathy involving renal papillary necrosis and chronic interstitial nephritis. Renal papillary necrosis is readily induced in experimental animals with combinations of aspirin and phenacetin (Molland, 1978; Kincaid-Smith, 1968). This lesson from the past has taught us that combinations of herbs or drugs may result in levels of toxicity that are not seen with one agent individually and that common combinations should have some preclinical testing performed. Other pharmacological safety studies using animals can be performed to investigate the potential undesirable pharmacodynamic effects of a single substance on physiological functions (Valentin *et al*., 2009). There is often very little known about the pharmacodynamics of a medicinal herb until such studies are performed. Animal studies lasting 4 weeks with subsequent histologic evaluation of organs may yield important information about insidious organ damage or inflammation that the herb or drug may cause (Greaves *et al*., 2004).

New, or different, extraction processes can yield different individual phytochemicals, which may affect efficacy

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and or toxicity. For instance, when garlic cloves are crushed into vegetable oil, the main product is diallyl trisulphide, whereas when crushed into water, the sulphur containing components convert to allysulphides (Wojcikowski *et al.*, 2007a). The difference in the final phytochemical makeup of garlic supplements is thought to be the reason for at least some of the discrepancy between results of human supplementation studies (Wojcikowski *et al.*, 2007a). In other instances, the polarity of the extraction solvent may result in the extraction of different constituents which may offer more, or less, benefit or toxicity (Wojcikowski *et al.*, 2007b). Well-planned combinations of *in vitro* and, when justified, *in vivo* animal testing of these extracts may help identify these differences prior to human trials and usage.

One of the most common types of herbal studies on animals is one that attempts to discover the mechanism by which the herb acts. Once discovered, this information can be valuable in determining whether the herb should be investigated for increasing the benefits of another herb or drug with known actions. For instance, in our own studies, we found that extracts of *Angelica sinensis* and *Astragalus membranceous* decreased renal fibrosis by decreasing fibroblast activation, collagen deposition and tubular apoptosis (Wojcikowski *et al.*, 2010). Since the main effects did not involve the renin-angiotensin mechanism, we hypothesized that the herbal extract combined with angiotensin converting enzyme inhibitors would provide more protection against renal fibrosis than either drug separately. We have found that this is true in experimental animals and await further studies to determine how this correlates to humans (Wojcikowski *et al.*, 2010).

DOSE TRANSLATION OF ANIMAL STUDIES

There have been numerous methods devised to predict drug effect based by body weight; however, the most accepted method is still based on surface area, as presented by DuBois and DuBois in 1915 (Du Bois and Du Bois, 1916; Dubois and Dubois, 1915). These authors postulated that the correct dose of a drug is proportional to the surface area in the two species rather than the body weight. In other words, larger animals generally require a smaller drug dose on a milligram per kilogram basis.

The history of this assumption began in 1883, when Rubner discovered that small animals utilized relatively more oxygen and produced more heat than larger animals (Rubner, 1883). Later, Dreyer and Ray studied blood volume of mammals, finding that while the ratio of blood volume to body weight in animals decreased with increasing animal weight, the relation of blood volume to BSA was constant (Dreyer and Ray, 1911). These early experiments led others to postulate about the evolution of various species. Since larger animals have less capacity for losing heat than smaller animals, they could have evolved new metabolic mechanisms that functioned at higher temperatures. Instead, the larger animal species retained similar anatomical features, but developed by functioning at a lower metabolic rate (Sharma and Mcneill, 2009).

While there has been no specific guidance for herbal medicines, the United States Food and Drug Administration (USFDA) has published a formula to calculate human equivalent dose (HED) of pharmaceuticals involving the species' K_m factors (body weight in kg divided by BSA in m^2) (USFDA, 2005) Their formula for dose translation is HED (mg/kg) = animal dose (mg/kg) × animal K_m/human K_m. This formula was presented to determine minimum recommended starting dose for drugs entering into clinical trials; however, it is still the most accepted guideline for animal to human dose conversion of biologically active constituents at this time (Sharma and Mcneill, 2009). Examples of how this relates to a number of different species are presented in Table 1, while examples of the error that is made by using body weight alone are

Table 1. US Food and Drug Administration guidelines for calculation of human equivalent dose

Species	Body weight (kg)	Body surface area (m^2)	K_m factor	Rapid calculation[a]
Human adult	60	1.62	37	
Mouse	0.020	0.007	3	12.3
Hamster	0.080	0.016	5	7.4
Rat	0.150	0.025	6	6.2
Ferret	0.300	0.043	7	5.3
Guinea pig	0.400	0.05	8	4.6
Domestic cat	2.5	0.25	10	3.7
Rabbit	1.8	0.15	12	3.1
Monkey	3	0.25	12	3.1
Dog	10	0.50	20	1.8
Pig	20	0.74	27	1.4
Gelding	540	6.4	84	0.45

Examples of body weight, body surface area (BSA) and K_m factors for use in calculation of human equivalent dose (HED). K_m factors are derived by dividing body weight in kg by BSA in m^2. The formula for dose translation recommended by the US Food and Drug Administration is HED (mg/kg) = animal dose (mg/kg) × (animal K_m/human K_m).(USFDA, 2005) Toxicity studies should include a safety factor of 10. This table is not to be used for calculating HED for children (refer to text).

[a]For rapid calculation of HED if the animal dose is known, divide animal dose in mg/kg by the number in this column. The result is in mg/kg.

[a]For rapid calculation of the animal dose if the human dose is known, *multiply* the mg/kg/day human dose by the number in the rapid calculation column and then multiply by the weight of the animal. A veterinarian should be consulted prior to administration of biologically active substances to pets.

Table 2. Examples of the differences made in dose conversion with isometric versus allometric scaling

Species	Animal dose (mg/kg)	Isometric scaling of dose to 60 kg human (mg)	Allometric scaling of same dose to humans (mg/kg)	Allometric scaling for a 60 kg human (mg)
Mouse	100	6000	8.130081	487.8049
Hamster	100	6000	13.51351	810.8108
Rat	100	6000	16.12903	967.7419
Ferret	100	6000	18.86792	1132.075
Guinea pig	100	6000	21.73913	1304.348
Cat	100	6000	27.02703	1621.622
Rabbit	100	6000	32.25806	1935.484
Monkey	100	6000	32.25806	1935.484
Dog	100	6000	55.55556	3333.333
Pig	100	6000	71.42857	4285.714
Gelding	100	6000	222.2222	13333.33

Example of the error made with isometric scaling (direct extrapolation on a mg/kg basis) of a 100 mg/kg dose from animals to humans. A 100 mg/kg dose in any species is extrapolated to 6000 mg in a 60 kg human if isometric scaling is used. Allometric scaling is preferred.

presented in Table 2. When safety is a major concern, the minimum anticipated biological effect level can be used, which is the lowest dose that produces a biological effect of any kind (Duff, 2006). For adverse effects of new medicinal herb extracts, a safety factor of at least 10 should be applied due to the possibility that humans may be more sensitive to the toxic effects of the extract, or that bioavailability of the active components of the herb may be higher in humans than the animal species (USFDA, 2005).

Sharma and McNeil have described drugs which may not be amenable to allometric scaling (Sharma and Mcneill, 2009). These include drugs that are highly protein bound, drugs that undergo extensive metabolism and active transport, drugs that undergo significant biliary excretion or renal secretion, drugs whose targets are subject to significant inter species differences and biological drugs that exhibit significant target-binding effects (Sharma and Mcneill, 2009).

Children

Isometric scaling should not be applied to children, as a child's dose of any substance is generally not a mg/kg fraction of the adult dose. In fact, adjusting dose from adults to children is often as challenging as scaling among species (Sharma and Mcneill, 2009). The processes of absorption, distribution, metabolism and excretion are all immature during infancy, making it potentially dangerous to administer unproven doses of medicinal herbs to those less than 18 months of age. For children older than 18 months, Johnson found that a specific rule involving BSA most reliable: child' s dose = adult dose x (BSA of the child/BSA of the adult) (Johnson, 2008). Another formula involving a variation of Clark's body weight was also found to be valuable: child's dose = [adult dose × (child's weight/adult's weight]$^{0.75}$ (Johnson, 2008). A number of authors state that scaling for children must only be used as a last resort, that extrapolation of dosage from animals to children should not be attempted and that doses should be carefully titrated according to response (Johnson, 2008; Lack and Stuart-Taylor, 1997; Sharma and Mcneill, 2009).

PREDICTABILITY

There are numerous arguments against the use of animal studies on medicinal herbs or drugs, many of which involve the fact that animal studies do not necessarily predict what will happen in humans (Fisher and Tatlisumak, 2005; Pound *et al.*, 2004). It is true that a significant interspecies variation in any of the components of pharmacokinetics or pharmacodynamics may result in inappropriate extrapolation of animal dose to humans. Another reason for concern is the lack of variability between subjects in animal studies, as the animals normally are young healthy adults that are of homogenous genetic background and are housed and fed uniformly (Olson *et al.*, 2000). Arguments against animal studies have also included the fact that only 37%–44% of animal studies published, that had more than 500 citations, resulted in human randomized trials (Hackam and Redelmeier, 2006; Ioannidis, 2006).

There has been no work drawing correlations from studies testing the benefits or toxicities of medicinal herbs from animals to humans. In fact, there have been few attempts to assess the correlation between the effects of drugs in animals and humans. From these studies, it is clear that there are numerous adverse drug reactions in humans that cannot be detected in animals (Greaves *et al.*, 2004). Although the preliminary analysis of these cases might suggest a poor correlation between non-clinical and clinical data (yielding poor predictive value of animal studies), Valentin and colleagues argue that in most cases, this can be associated with inappropriate, limited non-clinical testing and other factors not associated with the true concordance rate (Valentin *et al.*, 2009). For instance, hepatobiliary toxicity in humans was poorly predicted (50%) from animal studies (Olson *et al.*, 2000), but Greaves explained that this is, at least partly, because aminotransferase enzyme levels were often used, which are relatively insensitive markers of liver toxicity (Greaves *et al.*, 2004). Correlation with human toxicity was much better if the study incorporated histopathology data from animals (Greaves *et al.*, 2004). Greaves further argued that the true concordance level of hepatotoxicity would be higher simply because it is probable that new drugs that produce severe hepatotoxicity in animals are not tested in humans. Finally, another

reason for decreased predictability in some animal experiments was that they lacked ideal duration, as studies performed for less than two weeks have a poor predictive value for detection of hepatotoxicity. One month of animal studies followed by histopathological examination of the tissues to determine the expression of pathological change in tissues will detect 99% of the hepato-toxicities that can be detected in animal models (Greaves *et al.*, 2004).

Since animals have no ability to communicate symptoms, animal studies are often criticized for their inability to detect the most common adverse drug reactions including headache, anorexia, dizziness, sleepiness, oedema and flushes (Olson *et al.*, 2000). However, studies aimed at analysing correlations between symptoms in humans and signs in animals have found that dizziness correlates to spontaneous motor activity, oedema correlates with urinary sodium excretion, anorexia correlates to gastric emptying time and headache and malaise correlate to decreased blood pressure in experimental animals (Valentin *et al.*, 2009). Animal studies including the results of the signs just mentioned may have increased concordance rates to outcomes in humans (Valentin *et al.*, 2009).

While the overall concordance rate for animal to human studies was estimated to be about 70% (Olson *et al.*, 2000), avoiding previous mistakes should help increase the concordance (Greaves *et al.*, 2004). Concordance for the cardiovascular and hematopoietic systems appears to be the strongest (80% and 91%, respectively) (Macdonald and Robertson, 2009). The skin and hypersensitivity reactions show the least concordance between effects in animal studies and human patients (Greaves *et al.*, 2004). Unfortunately, at this time, it is unlikely that any combination of solely *in vitro* studies would reach the level of predictive accuracy seen with whole animal studies; however, there is continuing work in this area, and it is hoped that this work will result in the development of new and improved predictive technologies (Macdonald and Robertson, 2009).

PRACTICAL APPLICATIONS OF CONVERSION PRINCIPLES

A brief discussion of some of the recent animal studies on medicinal herbs will serve to highlight the practical applications of the conversion principles just discussed. In 2012, Khalili *et al.* found that 300 and 500 mg/kg dried extract of *Hypericum perforatum* (hypericum; St. John's wort) once every two days reduced the number of renal calculi in rats by 40% (Khalili *et al.*, 2012). The dried extract was originally obtained using an 80% ethanol: water solvent on the leaves of the plant. To determine the human dose equivalent, 150 mg/kg (the small average daily dose) is divided by 6.2 (as per Table 1), and the result (24.19) is multiplied by a 60 (for a 60 kg human), making the human dose equivalent for a 60 kg person 1451.61 mg/day. Clinical trials testing the extract on humans with depression have used doses of 500 to 1800 mg/day dose, and those trials were well tolerated with side effects (gastrointestinal disturbances, fatigue, dizziness, confusion, dry mouth) near placebo levels (Kasper *et al.*, 2010). Therefore, the study cannot be criticized for using the 'wrong' dose; however, it may have been of greater benefit to practitioners if the low dose in the animal experiment had been 50–75 mg/day (100–150 mg/every second day). It is often difficult for scientists to choose the optimal dose in animal experiments testing the benefits of herbs, because if too small a dose is used, there may be no observed effect, decreasing the likelihood of approval for further studies.

Perhaps, the compromise that future studies should consider is to test one dose that is equivalent to the lowest recommended dose for humans and one significantly higher to observe adverse effects and for the scientific community interested in drug discovery. A good example of the use of normal and high doses for determining the benefits of medicinal herbs is the study by Dost *et al.* (2009), who investigated the effect of a hypericum extract similar to that used by Kasper *et al.* (2010) on the inflammatory and immune response in rats with induced inflammatory bowel disease (Dost *et al.*, 2009). Rats received doses of 50, 150 and 300 mg/kg/day, which corresponds to HEDs of 483.871, 1451.613 and 2903.226 mg/day in a 60 kg human. The HED of the low dose in that study was near the 500 mg/kg low dose used in clinical trials for depression, while the largest dose (300 mg/kg in rats) was equivalent to over twice the high dose for humans, meaning that the beneficial effects observed in the animals may indeed be relevant to humans at relatively low doses. The safety profile of hypericum has been elucidated (Kasper *et al.*, 2010), so an even higher dose was not necessary.

There are several worthwhile points of discussion in the recent toxicity study on *Actaea racemosa* (black cohosh) (Mercado-Feliciano *et al.*, 2012). The recommended human dose for black cohosh dried extract is 40 mg per day, which is 0.57 mg/kg/day for a 70kg human or 0.66 mg/kg for a 60kg human. The researchers investigated the effects of a dried extract (obtained using a 50% aqueous ethanol extraction solvent) in rats and mice at doses of 0, 15 (rats only), 62.5 (mice only), 125, 250, 500 or 1000 mg/kg for 90 days. Table 3 extrapolates the HEDs of these animal doses, which are between 3.6 and 241 times (362 and 24193%) the recommended daily dose for a 60 kg human. The results indicated that liver weights were increased in the top two doses in mice and the top dose in rats. Two of the ten rats had mild liver necrosis with no changes in the liver function tests. This supports the recommendations by Greaves *et al.* (2004) who explain that liver enzyme biochemistry is not a reliable tool for evaluation of liver toxicity in animals and that histological samples should be always be evaluated (Greaves *et al.*, 2004). The study also found a dose-dependent non-regenerative normochromic macrocytic anaemia. The lowest dose that had any effect was 62.5 mg/kg in mice, which extrapolates to an HED of 7.6 or 8.9 times the recommended human dose in a 60 or 70 kg person, respectively. Since the dose at which changes began to occur is near the FDA recommendation of 10 times or higher for toxicity studies, the authors correctly concluded that pharmacokinetic studies of several marker components should be done to obtain a better animal to human comparison. However, given that relatively serious pathology did not occur until 121 and 242 times the equivalent of the recommended human dose, it is unlikely that the extract will be found to be dangerous. Finally, regarding

Table 3. Extrapolation (allometric scaling) of the human equivalent doses (HED)

Dose in mg/kg	Species	HED in mg/kg	HED (60 kg human)	% of 60 kg human dose	Isometric conversion of same dose (60 kg human)
15	Rat	2.419355	145.1613	362.9032	900
62.5	Mouse	5.081301	304.878	762.1951	3750
125	Rat	20.16129	1209.677	3024.194	7500
125	Mouse	10.1626	609.7561	1524.39	7500
250	Rat	40.32258	2419.355	6048.387	15000
250	Mouse	20.3252	1219.512	3048.78	15000
500	Rat	80.64516	4838.71	12096.77	30000
500	Mouse	40.65041	2439.024	6097.561	30000
1000	Rat	161.2903	9677.419	24193.55	60000
1000	12.3	81.30081	4878.049	12195.12	60000

Extrapolation (allometric scaling) of the HED in the study by Mercado-Feliciano *et al*. (2012) (29). Isometric conversion (direct extrapolation on a mg/kg basis; final column) is provided for comparison to the HED (60kg human). Isometric scaling is not recommended (refer to text).

the conversion made in that study, the authors state that 'in the current study the lowest dose that had an effect (62.5 mg/kg/day) was 125 times the currently recommended amount for daily consumption (≈ 0.5 mg/kg/day) for a 70 kg human'. This is an example of the mistake that is made when direct extrapolation on a mg/kg basis (isometric scaling) is used rather than the preferred method of scaling by allometry. Allometric scaling of the 62.5 mg/kg daily dose in mice yields 5.08 mg/kg in humans or 355.69 mg/day in a 70 kg human, which is 8.9 times the current recommended daily dose.

In another study, a dried extract of *Centella asiatica* (centella; gotu kola) was found to lack evidence of toxicity in mice at very high doses (Chauhan and Singh, 2012). The authors stated that extracts of centella possess antioxidant, cognitive-enhancing and antiepileptic properties. However, the studies cited were performed on extracts that used water as the extraction solvent, while the study by Chauhan and Singh (2012) used acetone as the solvent (Chauhan and Singh, 2012). There would be few (if any) overlapping constituents extracted by acetone when compared to those extracted by water (Wojcikowski *et al*., 2009), and therefore the results of the study do not relate to aqueous extracts of centella. Most studies use an aqueous-alcoholic extraction process. Since the polarity of an aqueous-alcoholic fluid is between the polarity of water and acetone, there is often some overlap in constituents (Wojcikowski *et al*., 2009). The yield in that study is also noteworthy, as the amount of material that was extracted with acetone was only 5.7% of the weight of the herb, meaning that the dried extract would be considered a 17.5:1 extract. This is can be important when considering dried solvent extracts relative to the dried plant equivalent. A dried whole plant product is considered 1:1 dry plant equivalent.

CONCLUSIONS

Animal studies on medicinal herbal extracts are not perfect. The unique evolution of each species is reflected by interspecies differences in protein binding, transport and metabolism of the active constituents in the pharmacokinetic phase and changes in receptor expression, affinity and distribution in the pharmacodynamic phase (Sharma and Mcneill, 2009). This will inevitably lead to some degree of error in extrapolation of results to humans, regardless of the conversion method used. However in many cases, animal studies can provide information that is not able to be provided by *in vitro* studies, computer modeling or even traditional use or human trials. Properly designed animal studies should carefully consider the appropriate dose using allometric scaling, the best animal model for the goals of the study and adequate duration of the study. Correlative data for potential symptoms and histological evaluation of tissues will provide the most information with the lowest number of animals. While there remains a poor correlation between cutaneous and hypersensitivity reactions, the effect on most organs is usually quite similar, and animal data can be used as additional scientific information that if cautiously interpreted, can increase the safety of medicinal herbs by guiding practitioners to make the most appropriate prescriptions.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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